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To the state secretary for
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DATE 27 February 2019

REFERENCE CGM/190227-01

SUBJECT Research report on the environmental risk assessment of the clinical use of GM T cells

Dear Mrs Van Veldhoven,

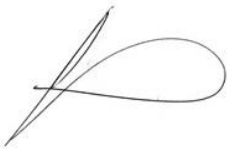
COGEM commissioned a literature study into the potential transfer of genetically modified (GM) T cells to third parties and the possible effects they may have in the recipient. The report on this research forms the basis for COGEM's observations.

Summary:
The amount of research into genetically modified (GM) T cell cancer therapies has been growing in recent years. In such therapies, T cells from the patient are genetically modified outside the body (ex vivo) to enable them to recognise cancer cells so that when they are returned to the patient they will fight the cancer. The environmental risk assessment of gene therapy studies includes consideration of the risks of possible third party exposure to the GMO. GM T cells can be transferred from the patient to third parties via needlestick injuries, donated organs, tissues or blood, across the placenta (to the unborn child), via mother's milk and via semen (sexual contact). To gain a better understanding of the levels of possible exposure and the potential risks, COGEM commissioned a literature study.

Based on the information in the research report and the scientific literature, COGEM observes that GM T cell therapy can, in specific cases, present a potential risk to third parties. The risk of exposure via needlestick injuries and via semen and donated blood is considered to be negligible. However, exposure to GM T cells via donated tissues, organs or stem cells, across the placenta and via breastfeeding can present a potential risk. The consequences can be serious, but are hard to predict and depend on the type of genetic modification to the T cell. More research is needed before any definitive statements can be made. In addition, a clear understanding is needed of which measures could be required to prevent transfer and where the responsibility for such measures should lie. COGEM observes that the relevant authorities and institutions should be aware of the possible risks and that patients eligible for GM T cell therapy should be informed of the possible risks in combination with pregnancy, breastfeeding and organ, tissue or stem cell donation.

The attached report contains COGEM's advice on this topic and a discussion of the underlying reasoning.

Yours sincerely,



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Immunotherapy with genetically modified T cells: unintended exposure and potential risks

COGEM Report CGM/190227-01

1. Introduction

In recent years there has been an increasing amount of research worldwide into the use of genetically modified (GM) T cell therapies for various forms of cancer. In such therapies endogenous T cells from the patient are genetically modified outside the body (ex vivo) to enable them to recognise cancer cells so that when they are returned to the patient they will fight the cancer. The standard environmental risk assessment of clinical gene therapy studies includes consideration of the risks of third party exposure.

GM T cells cannot survive outside the body and are not secreted in saliva, urine or faeces. However, GM T cells may be transferred from the patient to other people via needlestick injuries, via donated organs, tissues or blood, across the placenta (to the unborn child), via mother's milk and via semen (sexual contact). For the environmental risk assessment it is important to establish how long GM T cells remain in the patient, whether or not the GM T cells can in fact be transferred via the routes stated above and what risks are associated with exposure to GM T cells. To learn more about this COGEM commissioned a literature study, which is summarised in the research report: Milieurisico-analyse van klinische toepassingen van genetisch gemodificeerde T-cellen.

The research was carried out by H. Bergmans and R. Kleinjans of Ameco and J.H. van den Berg of the Netherlands Cancer Institute. As the volume of available experimental data on the transfer of GM T cells to third parties is minimal, the information in the report mainly concerns the transfer of unmodified T cells. Based on the information in the attached research report and a supplementary study of the scientific literature, COGEM is of the opinion that transfer of GM T cells to third parties cannot be ruled out. This present policy report examines the transmission routes and possible associated risks.

2. Genetic modification of T cells

Natural T cells contain a T cell receptor that recognises antigens, which gives them the ability to detect abnormal cells (e.g. xenobiotic, infected or malignant cells). Antigens can only be recognised by a T cell when they are presented on a human leukocyte antigen (HLA) complex by another cell. When the antigen binds with the T cell receptor, the T cell is activated and divides, setting in motion the immune response to kill the abnormal cells. The immune response can be manipulated by genetically modifying the T cell receptor or developing a comparable artificial chimeric antigen receptor (CAR) to recognise a specific antigen. In contrast to T cell receptors, CARs do not need the antigen to be presented on an HLA complex and can be programmed to target many different surface proteins.[3] There are various methods for bringing the CAR transgene to expression in the patient's T cells (e.g.

by electroporation of a plasmid or mRNA), but a much used and effective method is ex vivo transduction of the T cell by means of retroviral or lentiviral vectors.[1]

3. GM T cell therapy

GM T cells are a promising treatment method for various forms of cancer.[2] The best results so far have been obtained from treating blood (haematological) cancers, such as acute lymphatic leukaemia and non-Hodgkin lymphoma. In particular, the positive results obtained from an experimental GM T cell therapy targeting CD19 on malignant B cells has led to an increase in the number of clinical studies with GM T cells.[2,3] The average complete remission of (young) patients treated with anti-CD19 CAR T cells for acute lymphatic leukaemia was between 70% and 90%.[4] GM T cell therapies have also been developed that target solid tumours (e.g. neuroblastomas and sarcomas), but these have so far been less successful.[2,5]

GM T cell therapy also has potential as an antiviral therapy. A growing research topic in recent years is the control of HIV (human immunodeficiency virus), which following infection can remain latent in CD4+ T lymphocytes. [6,7,8] Research is also being conducted on the effectiveness of T cell therapies for other viral diseases, such as infections with hepatitis B virus, hepatitis C virus and human cytomegalovirus.[9,10,11]

4. Environmental risk assessment

Since 2011, COGEM has assessed increasing numbers of permit applications for clinical studies on the use of retroviral or lentiviral transduced GM T cells in immunotherapies for various types of cancer (predominantly B cell malignancies).[12,13,14,15,16,17,18,19,20] The potential adverse effects of GM T cell therapy on the patient have been widely studied. Because T cells cannot survive outside the body, transfer to third parties is limited to situations in which blood or bodily fluids containing T cells are exchanged. Needlestick incidents, transfer via the placenta or mother's milk and transmission via semen are possible routes for exposure to GM T cells. However, the potential adverse effects of unintentional exposure depend on various factors, such as the number of T cells the recipient is exposed to, the specificity of the GM T cell, the treatment strategy used and the immune status (or HLA type) of the recipient. The following sections describe in more detail the lifespan of GM T cells and the potential for transfer of GM T cells.

4.1 Lifespan of GM T cells

It is not easy to determine the lifespan of GM T cells. GM T cells can divide following activation by binding to the selected antigen. When exposed repeatedly to the antigen, GM T cells can persist for longer and in greater numbers. GM T cells can remain present for several months or even years after being administered in the initial treatment. Various studies show that GM T cells can be detected in patients for at least a year after being administered.[21,22,23,24,25,26,27] In one study GM T cells were detected 11 years after being administered, with an estimated half-life of more than 16 years.[28]

The characteristics of the antigen receptor or CAR may also have an influence on persistence. For example, CARs consisting of mouse antibody fragments can be killed more quickly by the body's own immune system than CARs consisting of human antibody fragments.[1] Given that immunotherapy has been an emerging form of treatment over the past two decades, no definitive statements can yet be made about the persistence of GM T cells during the life of the patient. Neither can detection in blood always provide conclusive evidence of the presence of T cells in the body, because these cells – like regular T cells – are contained in primary and secondary lymphoid organs or other organs. More long-term research is needed into the presence of GM T cells in blood and other tissues before any definitive statement can be made about persistence. For the time being, COGEM considers it plausible that after GM T cells are administered they are able to persist in the body for long periods, up to several years.

4.2 Transfer of autologous GM T cells

4.2.1 Needlestick incidents

Needlestick injuries and other incidents can cause medical staff treating patients to become contaminated with GM T cells. Although needlestick injuries would result in the injection of considerably fewer GM T cells than patients receive during treatment, just a single T cell transferred to the recipient, once activated by binding to the antigen, could divide and differentiate into various types of T cells.[29] If a healthy individual were exposed to modified T cells via a needlestick injury, however, there is a high probability that these cells would be immediately identified and eliminated by the recipient's immune cells. The cells would only be able to survive if the HLA molecules are completely identical to those of the patient (HLA compatibility). The chance of two non-related individuals being completely HLA compatible is exceptionally small because there are more than a million different haplotypes. In the unlikely event that the HLA molecules are identical and the GM T cells are tolerated by the needlestick accident victim, the GM T cells could have a similar effect to that in the patient. The presence or absence of the antigen that activates the GM T cells plays a major part in this. If the antigen is only present on the abnormal cells in the patient, any effects resulting from third party exposure will be negligible, because the recipient will not have any of these abnormal cells. If the target antigen of the GM T cell is also present in healthy cells, a third party exposed to the T cells could be adversely affected. However, given that HLA compatibility is extremely rare, COGEM considers the possibility of GM T cells surviving after being transferred to a third party via a needlestick injury to be negligible.

4.2.2 Transmission of GM T cells via semen

T cells make up just a small fraction of the leukocytes present in semen. The research report states that vaginal, rectal and oral transmission of cell-associated HIV/SIV or FIV (human, simian or feline immunodeficiency virus) have been observed in animal studies, from which it can be assumed that T cells in semen can be transmitted to third parties via the mucosa or via lesions. However, compatibility of the HLA haplotypes plays a part in this situation too. As HLA compatibility is extremely rare, the chances of any T cells surviving after a natural immune reaction are negligible. Consequently,

COGEM considers the chance of GM T cells surviving following transmission via semen to be negligible.

4.2.3 Transmission of GM T cells during pregnancy

During pregnancy cells are exchanged bidirectionally between mother and foetus. Some of the cells transferred from mother to child are T cells.[30] The research report states that an estimated 10^6 T cells can be transferred across the placenta to the unborn child. Because during pregnancy there is a mutual tolerance of cells from mother and foetus (HLA incompatibility is not harmful during pregnancy) the foetus does not trigger an immune response, despite the fact that half the genes (and antigens) of the unborn child come from the father.[31] Regulation of maternal immune cells is an important function during pregnancy.[32] Maternal cells can survive for a long time in the foetus and for long periods thereafter, even into adulthood.[33,34] It is not fully known why the maternal immune tolerance of the child persists for so long.[34] Neither is it known if GM T cells display a similar tolerance. No research has been carried out into the transfer of GM T cells from mother to child, but COGEM considers it highly likely that GM T cells can be transmitted via the placenta to the unborn child.

4.2.4 Transfer of GM T cells via mother's milk

Mother's milk is a vital source of nutrition for the newborn baby, but it is also crucial for the development of the child's immune system. Besides antibodies, leukocytes, including T cells, are transferred to the child via mother's milk. The largest numbers of lymphocytes are found in the colostrum (the first fluid produced by the mammary glands at the end of pregnancy). The numbers fall off during the subsequent weeks.[35,36] The research report contains a list of experimental animal models that have been used to show that T cells can be transferred via mother's milk. T cells can be absorbed across the gut epithelium of the newborn, which is highly permeable for a few days after birth.[36] Even when the gut is no longer permeable, leukocytes are still able to pass through the gut epithelium by transcytosis and enter the bloodstream. The possibility of GM T cells being transferred in this way to children breastfed by patients cannot therefore be ruled out. The research report states that a baby can be exposed to an estimated 10^7 T cells via mother's milk, but it is not clear how many of these pass through the gut epithelium. Taking all these facts into consideration, COGEM considers it possible that GM T cells can be transferred to the child via mother's milk.

4.2.5 Transfer of GM T cells via solid organ and tissue transplantation

If blood, organs or tissue from patients who have undergone a GM T cell therapy are later donated, they may contain GM T cells that can be transferred to the recipient. The Dutch Transplant Foundation (*Nederlandse Transplantatie Stichting*) applies certain criteria for the donation of organs (heart, liver, kidney, lung, pancreas and small intestine) and tissues (skin, blood vessels, ocular tissue, heart valves, bone tissue, cartilage and tendons). Certain groups of people are in principle not permitted to donate organs: cancer patients or former cancer patients (with the exception of a few non-metastatic or curatively treated tumours) and people with active viral infections.[37] Fewer restrictions apply to tissue donation, which is only not permitted after premalignant or malignant haematological conditions (in the past or at death) and after a proven metastatic melanoma. From this it can be concluded that not all

cancer patients, or former cancer patients, are excluded from organ or tissue donation. Patients with viral infections are subject to less stringent conditions.

Because solid organ and tissue transplantations involve the transfer of T cells (including GM T cells if present) and these transferred leukocytes can cause damaging immune reactions (graft versus host disease), donor tissues and organs are now subject to leukocyte depletion before transplantation. The methods used for this, however, are not equally effective in removing all T cells.[38,39] Standard practice in organ transplantation is perfusion (the passage of fluid through the organ) to keep the organ viable, which removes some of the leukocytes. In conclusion, COGEM cannot rule out the possibility of GM T cells being transferred via solid organ or tissue transplantation.

4.2.6 Transfer of GM T cells via donor blood

Cancer patients and former cancer patients are not permitted to donate blood for use in blood products (a condition set by Sanquin, the organisation responsible for blood supply in the Netherlands). The only exceptions to this rule are patients with a certain form of skin cancer or cervical cancer. Candidates with viral infections (including HIV, hepatitis B, C and E, and HTLV) are also excluded from donating blood. Since 2002, Sanquin also requires all standard cellular blood products to be leukocyte depleted.[40] This is because leukocytes can induce immune reactions and potentially serve as vectors for various viruses. Leukocytes, including T cells, are removed from blood products by filtration, leaving fewer than $5x10^6$ leukocytes per unit present in the product. In addition, for immunocompromised recipients and in cases of HLA compatibility, blood products are also irradiated to destroy any remaining vital T cells. COGEM considers that with the standard procedures for blood transfusion (leukocyte depletion and irradiation in cases of HLA matching[a] and immune incompetence) the chances of GM T cells surviving following transfer via blood transfusion in the Netherlands are negligible.

4.2.7 Transfer of GM T cells via stem cell donation

The products used for stem cell donation also contain T cells. When stem cells are donated for the treatment of haematological malignancies, the T cells should destroy the abnormal blood cells of the patient (graft versus tumour effect) and so T cell depletion is inappropriate. Stem cell donation is also subject to the condition that the donor has never been diagnosed with any form of cancer. However, this is also subject to exceptions: people with a basal cell carcinoma or an early stage of cervical cancer are not excluded from donating stem cells.

To limit immune reactions to transplanted stem cells, tissues or organs and to reduce the chances of graft versus host disease, HLA matching is used in a number of cases and the immune system of the recipient is suppressed (sometimes for the rest of their lives). In these cases, any remaining GM T cells transferred with the transplanted organ or tissue will not be destroyed by the recipient's immune

[a] This aims to achieve the best possible HLA compatibility.

system. Based on the above, it is highly likely that GM T cells can be transferred via stem cell donation.

4.3 Effects of transfer

4.3.1 Harmful effects of GM T cells

Transfer of GM T cells is a potential risk during stem cell donation and tissue and organ transplantation as well as during pregnancy (transfer across the placenta) and breastfeeding. T cells that have been transferred can persist for long periods of time. Because of this persistence, treatment with GM T cells at an early age can have consequences for a pregnancy later in life. Women treated at a young age with GM T cell therapy face the possibility of GM T cells being transferred to the unborn child during a pregnancy, who may suffer harmful effects as a result. Recognition of the antigen in the unborn child could lead to activation of the GM T cells and may induce a stronger immune reaction. The exact consequences of GM T cell activation on the development of the child are as yet difficult to estimate, because no research has been conducted on this topic.

Harmful effects that can occur following exposure to GM T cells have so far only been described for the patient. Besides having the intended antitumour or antiviral (on-target) effect, the GM T cells – depending on the antigen targeted by the CAR – may also destroy healthy cells (on-target, off-tumour effect). An example of this is the much used anti-CD19 CAR T cell therapy for B cell malignancies. The CD19 transmembrane protein is involved in the signal transduction via the B cell receptor and is expressed in B cells from early development until after differentiation in a plasma cell. CD19 is not found in pluripotent blood stem cells and in other tissues. Anti-CD19 GM T cells do not distinguish between malignant and healthy B cells, because the CD19 antigen is present in both cases. A potential side-effect of this is B cell depletion, resulting in a severe reduction in the number of B cells in the patient. Continual depletion of B cells is accompanied by an increased susceptibility to infection. Patients are then given immunoglobulins to prevent infection. To what degree this can occur in the unborn child, resulting in insufficient development of the immune repertoire and B cell deficiency, is hard to predict.

Other effects associated with the working of GM T cells include immune reactions against GM T cells, harmful effects resulting from insertional mutagenesis, uncontrolled cell division as a result of antigen activation, temporary neurotoxicity and cytokine release syndrome (CRS), which is caused by the enhanced immune response that is induced and the associated release of cytokines. In serious cases, CRS can lead to shock and organ failure.

The presence of the antigen targeted by the GM T cell in healthy tissue is a major factor in the occurrence of side-effects resulting from third party exposure. It is common practice to choose target antigens that are more highly expressed in tumour tissue, but it has so far proved difficult to find a tumour-specific antigen that is not found on healthy tissue. The receptors of the GM T cell (T cell receptor or CAR) are developed for a single antigen (the target antigen), but in exceptional cases the

GM T cells may also target a second antigen present on healthy cells. Such off-target effects are often only discovered after the treatment has ended and they remain hard to predict.

The harmful effects on the unborn child or newborn exposed to GM T cells can be different from the effects on the patient. A considerable number of the tumour antigens targeted by GM T cells are embryonal proteins that are less highly expressed in healthy cells of adults. If a GM T cell therapy against embryonal proteins has no harmful (on-target, off-tumour) effects on the patient, it may still have consequences for an unborn child or newborn resulting from the expression of these antigens in early development. These consequences are also hard to predict and more research is needed.

4.3.2 Effects of insertional mutagenesis

Transduction of T cells with a retroviral or lentiviral vector ensures that the transgene (with the CAR sequence) integrates stably into the DNA of the T cells (also called insertional mutagenesis). Both retroviral and lentiviral vectors have a semi-random integration pattern. If the transgene integrates in close proximity to another gene it may affect the activity of the other gene. For example, integration of the transgene near to an oncogene or proto-oncogene can raise the level of gene expression to the point that it can lead to uncontrolled cell division. One of the best known examples of this is the treatment of patients with the serious immune disorder X-linked severe combined immunodeficiency, in which haematopoietic stem cells are transduced with a gamma-retroviral vector. Five of 12 patients developed T cell leukaemia two to six years after treatment. In the first instance, harmful effects resulting from insertional mutagenesis present a risk to the patient, but the possibility of 'rogue' GM T cells being transferred to third parties before the effects on the patient are discovered cannot be ruled out.

However, insertion of the transgene is not necessarily always accompanied by changes in the regulation of gene expression elsewhere in the genome. Neither do changes in gene expression always have to be biologically relevant, or have a harmful effect. For example, in one study clonal expansion of an ex vivo retroviral transduced bone marrow cell was observed in which the patient benefited from this side-effect.[41] Additional mutations are often needed before an unfavourable insertion can lead to malignancy.[42] So far, harmful effects resulting from insertional mutagenesis have only been observed in studies with retroviral transduced cells. The chance of harmful effects is also greater when less differentiated cell types are used, such as haematopoietic stem cells, in contrast to adult T cells.[42] No negative effects resulting from insertional mutagenesis have been reported when lentiviral vectors are used, partly because the chance of integration of transgenes into promotor regions is lower when using lentiviral transduction.[43] New developments, such as the use of self-inactivating (SIN) vectors, can also reduce the chances of harmful effects.[44] Nevertheless, the chance of harmful effects resulting from insertional mutagenesis, even with the use of SIN vectors, cannot be ruled out entirely.

5. Management strategies

In clinical studies, management strategies are also used to quickly suppress any adverse effects of GM T cells. For example, the transgene can be modified in such a way that the GM T cells can be

deactivated. If a gene that can function as a binding domain for certain antibodies is inserted into the CAR sequence, the GM T cells can be selectively deactivated by administering these antibodies. Such 'kill switches' or 'suicide switches' can provide a good means of destroying GM T cells, not only in the patient, but also in anyone accidentally exposed to the GM T cells. However, it should be pointed out that it is extremely difficult to identify any adverse effects of GM T cells in the unborn child and that it is almost impossible to carry out an immediate in utero switch therapy.

A few strategies may be considered for limiting transfer of T cells via breastfeeding. Freezing and pasteurisation can kill all or most of the immune cells in mother's milk and severely reduce the activity of any remaining cells.[45,46] Pasteurisation of mother's milk is often used to limit the transfer of bacteria to the child. The most commonly used method of pasteurisation is Holder pasteurisation (heating to 62.5°C for 30 minutes), which kills all lymphocytes.[47]

6. Observations

Treatment with GM T cells is a promising new therapy. Much research is being conducted into new applications, such as the treatment of other cancers and antiviral therapies. In 2016 two GM T cell therapies were authorised for use in the European Union (Yescarta and Kymriah). Both therapies are directed against the CD19 antigen and are often used successfully to treat acute lymphocytic leukaemia and diffuse large B cell lymphoma.

COGEM observes that the transfer of GM T cells following GM T cell therapy can in specific cases present a potential risk to third parties. The expected effects following transmission via needlestick injuries and via semen and blood transfusions are considered to be negligible because the transferred GM T cells will be destroyed by the recipient's immune system. However, it cannot be ruled out that GM T cells transferred via tissue or organ transplantation, breastfeeding or across the placenta will have an adverse effect in the recipient.

Many disorders for which GM T cell therapies are under development (such as haematological malignancies, solid tumours and HIV, hepatitis B and C infections) are subject to exclusion criteria for the donation of tissue, organs, blood and stem cells. However, it cannot be ruled out that new GM T cell therapies will soon be developed for clinical presentations which present no obstacles to the donation of blood products, tissues or organs.

The consequences of exposure to GM T cells via transfer across the placenta (unborn child), breastfeeding (newborn) or by transplantation of tissues, organs or stem cells remain very hard to predict. They may be comparable to the effects that occur in the patient, but they also depend on the specific modification of the receptor on the GM T cell. For the time being it cannot be ruled out that a recipient will experience a damaging effect, which means that the decision on which additional containment and control measures will be needed to safeguard human and environmental safety will have to be made on a case by case basis.

Possible effective risk management measures are obligations on the patient never to become pregnant, not to breastfeed or donate mother's milk, or to donate tissues or organs. The degree to which these measures can be imposed or are proportional to the risks involved, and the appropriate ethical norms that should be applied, needs further investigation. This must include a clear understanding of which measures can be imposed to prevent transfer and where the responsibility for any additional measures lies. Quesitons to be answered are: does this issue fall within the scope of the GMO legislation, and does responsibility lie with the attending physician, the patient or the institutions responsible for donations and transplantation? It is essential that all the institutions involved come together and discuss this.

COGEM observes that the institutions involved must be aware of the potential risks and must take these into consideration in any decisions they take. In addition, patients eligible for GM T cell therapy should be informed of the possible risks associated with pregnancy, breastfeeding and tissue, organ and blood donation. Further, more research is needed into the consequences of exposure to GM T cells and research programmes for the development of GM T cells should receive additional funding for appropriate safety studies.

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